APPLICATION OF RAPD MARKERS FOR DISEASE RESISTANCE BREEDING IN BEANS.
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Breeding for disease resistance has employed a number of different strategies involving the use of single gene resistance, to the accumulation of multiple disease resistant traits into breeding lines which in many cases are lost in segregating populations derived from crosses with disease susceptible lines in plant breeders nurseries. Stavely et al (8) has indicated that obtaining stable rust resistance in beans may necessitate the pyramiding of broadly effective specific resistance genes into traditional cultivars. The strategy of gene pyramiding is appealing when one considers that the pyramid of the three rust resistance genes Up-2 with B-190 with Ur-3 affords resistance to 63 of the 65 rust races characterized in the USDA collection (Stavely p.c.). Only races 58 and 67 overcome this pyramid. Support for gene pyramiding in spring wheat has been demonstrated by Schafer and Roelfs (6) where no significant stem rust epidemic has occurred in N. America since 1955 since current spring wheat cultivars are known to possess as many as six resistance genes. It is understandable that single resistance genes have not provided durable resistance to a highly variable rust pathogen but a cultivar with four or five genes conditioning resistance to all races might remain resistant for years if the gene-for-gene concept is valid.

In beans, breeders have the opportunity to exploit the complimentation of Andean and Mesoamerican resistance genes and the duplicity of different resistance genes from within the same gene pools. For example the Andean Up-2 gene is complementary with the Mesoamerican Ur-3 gene, while the Mesoamerican B-190 gene partially duplicates the action of the Ur-3 gene when one considers the individual rust races each controls. However the procedure of gene pyramiding is not practical for plant breeders because of the epistatic interaction between resistance genes. Since the theory requires that both epistatic and hypostatic genes be combined into a single genotype, breeders have no convenient way to select for the hypostatic gene without using multiple inoculations with different rust races or test-crossing back to a susceptible genotype. A similar situation is now occurring in the breeding for resistance to bean common mosaic virus (BCMV). As breeders incorporate the epistatic bc-3 resistance gene into a range of different genotypes, the dominant I gene becomes hypostatic which prevents breeders from identifying and selecting the more desirable Ibc-3 recombinants from the single bc-3 carriers. The Ibc-3 combination affords pyramided resistance to all known strains of BCMV.

In an effort to achieve these objectives the strategy of marker assisted selection (MAS) offers the breeder a viable alternative to developing cultivars with pyramided resistance genes. The identification of RAPD markers tightly linked to resistance genes allows selection of the resistance gene indirectly since the expression of the molecular marker is not masked by any epistatic interactions which commonly occur between resistance genes.

In our lab at MSU we have successfully used the PCR protocol to identify four RAPD markers linked to three rust resistance genes. Near isogenic lines (NILs) were developed for the Up-2, B-190, Ur-3 rust resistance genes either through backcrossing or selection of late generation heterogeneous plants. Bulked samples of DNA of resistant and susceptible plants were pooled separately and screened against random decamers (Operon primers) using DNA polymerase (Stoffel Fragment) in a Perkin Elmer 480 thermal cycler employing a range of temperatures/times and cycles from 34 to 47 depending on the primer.

Miklas et al (5) were successful in identifying the first RAPD marker $OA14_{1100}$ tightly linked to the Up-2 gene in beans (no recombinants); Haley et al (4) identified the $OF10_{970}$ marker linked at 2.15cM from the B-190 and the $OI19_{460}$ marker tightly linked to the same B-190 gene block (no recombinants); Haley

et al (1) identified the $OK14_{620}$ marker at 2.23cM from the Ur-3 gene. Three way crosses designed to combine all three resistance genes into a single genotype have been made and the RAPD markers will be used to pyramid the three resistance sources. With the identification ((Stavely et al (7)) of more broadly based resistance genes in the PI collection further gene pyramiding of these hypostatic genes is now possible with these new sources. Gene pyramiding using MAS now allows us to ensure the durability of the Ur-3 gene which has been used extensively in our breeding program at MSU.

A similar strategy was employed to identify markers linked to the BCMV resistance genes. Haley et al (2) identified a coupling phase marker $OAD19_{690}$ at 1.9cM from the bc-3 gene and a repulsion phase marker $OS13_{660}$ at 7.1cM from the same gene and demonstrated the efficiency of repulsion markers over coupling markers in terms of selection efficiency. In addition Haley et al (3) identified the $OW13_{690}$ linked at 1.3 to 5.0cM from the I gene depending on which mapping population and I gene source was being studied. The I gene marker was detected in both Mesoamerican and Andean germplasm with and without linkage to the B locus and it has been used in our breeding program to identify Ibc-3 recombinants in BC_4F_3 derived populations of pinto and great northern beans where both genes were independently segregating.

RAPD markers are a new and exciting technology that afford breeders another approach to select for durable disease resistance where traditionally both the techniques and opportunities were limited.

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